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Rationally Designed Vaccines Targeting the V2 Region of HIV-1 gp120 Induce a Focused, Cross-Clade-Reactive, Biologically Functional Antibody Response

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ABSTRACT

Strong antibody (Ab) responses against V1V2 epitopes of the human immunodeficiency virus type 1 (HIV-1) gp120 envelope (Env) correlated with reduced infection rates in studies of HIV, simian-human immunodeficiency virus (SHIV), and simian immunodeficiency virus (SIV). In order to focus the Ab response on V1V2, we used six V1V2 sequences and nine scaffold proteins to construct immunogens which were tested using various immunization regimens for their ability to induce cross-reactive and biologically active V2 Abs in rabbits. A prime/boost immunization strategy was employed using gp120 DNA and various V1V2-scaffold proteins. The rabbit polyclonal Ab responses (i) were successfully focused on the V1V2 region, with weak or only transient responses to other Env epitopes, (ii) displayed broad cross-reactive binding activity with gp120s and the V1V2 regions of diverse strains from clades B, C, and E, (iii) included V2 Abs with specificities similar to those found in HIV-infected individuals, and (iv) remained detectable ≥1 year after the last boosting dose. Importantly, sera from rabbits receiving V1V2-scaffold immunogens displayed Ab-dependent cellular phagocytosis whereas sera from rabbits receiving only gp120 did not. The results represent the first fully successful example of reverse vaccinology in the HIV vaccine field with rationally designed epitope scaffold immunogens inducing Abs that recapitulate the epitope specificity and biologic activity of the human monoclonal Abs from which the immunogens were designed. Moreover, this is the first immunogenicity study using epitope-targeting, rationally designed vaccine constructs that induced an Fc-mediated activity associated with protection from infection with HIV, SIV, and SHIV.

IMPORTANCE

Novel immunogens were designed to focus the antibody response of rabbits on the V1V2 epitopes of HIV-1 gp120 since such antibodies were associated with reduced infection rates of HIV, SIV, and SHIV. The vaccine-induced antibodies were broadly cross-reactive with the V1V2 regions of HIV subtypes B, C and E and, importantly, facilitated Fc-mediated phagocytosis, an activity not induced upon immunization of rabbits with gp120. This is the first immunogenicity study of vaccine constructs that focuses the antibody response on V1V2 and induces V2-specific antibodies with the ability to mediate phagocytosis, an activity that has been associated with protection from infection with HIV, SIV, and SHIV.

Nonneutralizing antibodies (Abs) can protect against various viral infections, contributing to protection from alphaviruses, flaviviruses, respiratory syncytial virus (RSV), and cytomegalovirus, among others (reviewed in references 1 and 2). While the specificity and affinity of nonneutralizing Abs are dependent on the Fab fragment of Abs to target virions and infected cells, many of the biologic activities of these Abs are a function of the Fc fragment. Such activities include Ab-dependent cellular cytotoxicity (ADCC), Ab-dependent cellular phagocytosis (ADCP), Ab-dependent cell-mediated virus inhibition (ADCVI), complement activation and fixation, degranulation, and the release of proinflammatory cytokines (3). Specific examples include protection from herpes simplex virus 2 in mice by nonneutralizing Abs which mediate ADCC (4–6) and protection from influenza virus in mice by nonneutralizing Abs targeting the head or stalk of the influenza virus hemagglutinin (7, 8).

Fc-mediated nonneutralizing Ab functions also play a role in reducing and preventing infection with simian immunodeficiency virus (SIV), simian-human immunodeficiency virus (SHIV), and human immunodeficiency virus (HIV) and in controlling virus replication *in vivo* (9–18). As early as 2004, vaccine-elicited Abs were shown to mediate ADCC, which correlated with reduced acute viremia in rhesus macaques challenged with SIV_{mac251} (19, 20). Subsequently, a myriad of studies have shown that nonneutralizing Abs mediating ADCC and other Fc-dependent activities can prevent or contribute to control of SIV (21–23) and SHIV

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TABLE 1 Sequence of V1 and V2 variable loops in immunogens and antigens[a]

Strain (clade)	V1 sequence	V2 sequence
1086 (C)	CVTLNCTNVKGNESDTSEVMKN	CSFKATTELKDKKHKVHALFYKLDVVPLNGNSSSSGEYRLINC
A244 (E)	CVTLHCTNANLTKANLTNVNNRTNVSNIIGNITDEVRN	CSFNMTTELRDKKQKVHALFYKLDIVPIEDNNDSSEYRLINC
92TH023 (E)	CVTLNCTNANVTNVKNITNVPNIIGNITDEVRN	CSFNMTTELRDKKQKVHALFYKLDIVPIEDNTSSSEYRLINC
Case A2 (B)	CVTLNCIDLRNATNATSNSNTTNTTSSSGGLMMEQGEIKN	CSFNITTSIRDKVQKEYALFYKLDIVPIDNPKNSTNYRLISC
YU2 (B)	CVTLNCTDLRNATNTTSSSWETMEKGEIKN	CSFNITTSIRDKVQKEYALFYNLDVVPIDNASYRLISC
ZM109 (C)	CVTLNCTSPAAHNESETRVKH	CSFNITTDVKDRKQKVNATFYDLDIVPLSSSDNSSNSSLYRLISC
CAP45 (C)	CVTLRCTNATINGSLTEEVKN	CSFNITTELRDKKQKAYALFYRPDVVPLNKNSPSGNSSEYILINC
ZM53 (C)	CVTLNCSKLNNATDGEMKN	CSFNATTELRDKKKQVYALFYKLDIVPLDGRNNSSEYRLINC
cV2[a] (E)		CSFNMTTELRDKKQKVHALFYKLDIVPIEDNTSSSEYRLINC

[a] Cyclic $V2_{92TH023}$ peptide.

(23–26). Significantly, Abs that mediate ADCC have been found in human breast milk and correlate with reduced risk of transmission from mother to infant (27).

Several streams of data from the RV144 clinical vaccine trial also suggest that reduced rates of HIV infection are associated with Abs that mediate ADCC and ADCP, but, notably, no correlation with the presence of serum neutralizing Abs was identified in RV144 vaccinees (10–18). The protective role for Fc-mediated Ab function is further supported by the finding that specific alleles of FcγRIIc (which carries the extracellular sequence of FcγRIIa, a critical participant in ADCP) were associated with RV144 vaccine efficacy (10). Moreover, the V2-specific IgG Ab levels, which were an independent correlate of a reduced infection rate in RV144 vaccinees (13, 28–31), appear to have played a role in the Fc-mediated vaccine-induced antiviral activities in that V2 Abs contributed to ADCC responses (14, 16) and were found to synergize with constant region 1 (C1)-specific Abs that were also induced by the vaccine (11). V2-specific Abs have also been identified as correlates of protection after immunization and challenge with SIV (32–34).

Given these findings in monkeys and humans, we hypothesized that targeting the Ab response to V2 with rationally designed immunogens would improve the functional Ab quality and provide a basis for enhanced efficacy of HIV immunization protocols. In the work described below, we tested the immunogenicity of 12 V1V2-scaffold protein immunogens. The results show the induction of V2-specific Abs that are reactive with V1V2 and with gp120 Envs from diverse strains and clades. After priming with gp120 DNA with or without V1V2-scaffold protein immunogens and boosting with V1V2-scaffold proteins, a durable Ab response was successfully focused on the V1V2 region. This is the first immunogenicity study using rationally designed V1V2-targeting vaccine constructs. Similar to the results of previously published studies with V3-scaffold immunogens (35, 36), the V1V2-scaffold immunogens induced Abs that recapitulate the specificity and activities of the human V1V2-specific monoclonal antibodies (MAbs) whose epitopes were used as templates for the design of the V1V2-scaffold immunogens (37); importantly, the induced Abs display a biologic activity—in this case, Fc-mediated ADCP—which has been associated with protection from infection with HIV, SHIV, and SIV (12, 18, 24, 38).

MATERIALS AND METHODS

Use of a codon-optimized HIV Env DNA, recombinant V1V2-scaffold proteins, and various protein and peptide antigens. Codon-optimized gp120 DNA expressing *env* from HIV clade C primary isolate ZM109F (39) was prepared in the pJW4303 vector with a tissue plasminogen activator (tPA) leader sequence, as described previously (40, 41). The genes of the following proteins were used to prepare the scaffolds of the V1V2-scaffold immunogens: PDB accession number 1FD6 (42), typhoid toxin subunit B (TTB) (43), and PDB accession numbers 2J9C (44) and 2F5K (45). The V1V2 sequences of the full-length V1V2 regions used as inserts into the scaffolds are listed in Table 1. Genes of the V1V2 scaffolds were chemically synthesized and cloned into the pVRC8400 plasmid, followed by expression in HEK293 $GnTI^{-/-}$ cells and purification by affinity chromatography (46). Details of the design and construction of the immunogens are described separately (47). The V1V2-tags protein of strain 1086 ($V1V2_{1086}$-tags) and $V1V2_{A244}$-tags were provided by H. Liao (Duke University), $V1V2_{CaseA2}$-gp70 and $V1V2_{92TH023}$-gp70 were provided by A Pinter, and $gp120_{A244}$ was provided by Global Solutions for Infectious Diseases (South San Francisco, CA). $gp120_{BaL}$, $gp120_{ZM53}$, and $gp120_{ZM233}$ were purchased from Immune Tech (New York, NY), and a clade C V3 consensus linear, nonbiotinylated 23-mer peptide (NNTRKS IRIGPGQTFYATGDIIG) and the cyclic $V2_{92TH023}$ ($cV2_{92TH023}$, clade E) biotinylated peptide were purchased from BioPeptide (San Diego, CA). A C5 linear nonbiotinylated 22-mer peptide (amino acids 495 to 516 of gp120, KIEPLGVAPWKAKRRVVQREKR) was purchased from PolyPeptide Group (Torrance, CA).

Immunization protocol. Female New Zealand White rabbits, 6 to 8 weeks old (with a body weight of ~2 kg), were purchased from Harlan Laboratories (Indianapolis, IN) and housed in the animal facility managed by the Department of Animal Medicine at the University of Massachusetts Medical School in accordance with an IACUC-approved protocol. Three to five rabbits were included in each immunization group. All rabbits received three DNA immunizations using a Bio-Rad Helios gene gun (Bio-Rad Laboratories, Hercules, CA). The gp120 DNA vaccine plasmids were coated onto 1.0-μm gold beads at a ratio of 2 μg of DNA per mg of gold. Each gene gunshot delivered 1 μg of DNA into a total of 36 nonoverlapping sites on the shaved abdominal skin of each rabbit at each of the three priming immunizations. Several groups of rabbits received protein immunogens simultaneously with the three DNA priming immunizations as described below; in addition, all animals received two protein boosts. Each dose of protein immunogen was delivered as a bolus of 100 μg intramuscularly together with adjuvant. Blood was collected prior to immunization and 2 weeks after each immunization. Rabbits were bled periodically for up to 76 weeks after the initiation of the immunization protocol to assess the longevity of the Ab response. The immunization schedule for each of four rabbit experiments is shown in Table 2.

Enzyme-linked immunosorbent assay (ELISA) method. Immulon 4 plates were coated using 100 μl/well of the designated antigen at a concentration of 1 μg/ml in carbonate buffer, pH 9.6, and incubated overnight at 4 °C. Plates were then washed six times with phosphate-buffered saline (PBS)–0.05% Tween. Subsequently, 100 μl/well of rabbit serum was added to the plates after dilution in RPMI medium containing 15% fetal bovine serum. Plates were incubated for 1.5 h at 37°C. After six washes, bound Abs were detected by the addition of 100 μl/well of a

TABLE 2 Immunization schedule

Week no.	Expt H1	Expt H2	Expt H3	Expt H4
0	DNA prime 1	DNA prime 1	DNA prime 1	DNA prime 1
2	DNA prime 2	DNA prime 2	DNA prime 2	
4	DNA prime 3	DNA prime 3	DNA prime 3	DNA prime 2
6				
8				DNA prime 3
10	Protein boost 1	Protein boost 1	Protein boost 1	
12				
14	Protein boost 2	Protein boost 2	Protein boost 2	Protein boost 1
16				
18				Protein boost 2

1:2,000 dilution (in PBS–0.05% Tween) of alkaline phosphatase-conjugated mouse anti-rabbit IgG (Southern Biotech, Birmingham, AL) and incubated for 1.5 h at 37 °C. After six washes, 100 μl/well of 10% diethanolamine substrate was added, and after 30 min, the plates were read at 405 nm using a Sunrise Tecan microplate reader equipped with Magellan 6 software. All samples were tested in duplicate. Various V2 MAbs were used as positive controls, and MAb 1418, specific for parvovirus B19, was used as a negative control in each experiment. For assessment of MAb reactivity, the same method was employed, using 10 μg/ml of MAb and alkaline phosphatase-conjugated goat anti-human IgG (Southern Biotech) as the secondary Ab.

Measurement of Ab-dependent cellular phagocytosis. Using the assay developed by Ackerman et al. (48), 10 μg of biotinylated $cV2_{92TH023}$ was conjugated to fluorescent NeutrAvidin beads (Invitrogen) according to the manufacturer's instructions. In addition, 20 μg of $gp120_{ZM53}$ (Immune Technology) or $V1V2_{YU2}$-1FD6 was biotinylated using EZ-Link sulfo-NHS-LC-LC-biotin (sulfosuccinimidyl-6-[biotinamido]-6-hexanamido hexanoate; Thermo Scientific), and these reagents were also conjugated to fluorescent NeutrAvidin beads. Conjugated beads were washed and resuspended in 0.1% bovine serum albumin (BSA)-PBS to a working dilution of 1:100. A total of 9×10^5 beads were aliquoted per well in round-bottom 96-well plates. Fourfold dilutions of a MAb or 2-fold dilutions of individual serum samples were added, incubated for 2 h at 37°C, and washed. A total of 2.5×10^4 THP-1 cells (ATCC) were added to each well and incubated overnight. Phagocytosis was measured by flow cytometry. ADCP scores were calculated as follows: (percentage of bead-positive cells × MFI of bead-positive cells)/10^6, where MFI is mean fluorescence intensity.

RESULTS

Immunogenicity study design. Eighteen different vaccination regimens were performed during the course of four rabbit experiments (H1 to H4) (Table 3) using 95 rabbits to test the immunogenicity of 12 vaccines constructed with six V1V2 sequences presented on one of nine protein scaffolds and administered intramuscularly with one of two adjuvants. Each immunization regimen was tested in three to five animals. All groups (with the

TABLE 3 Immunization protocol and ELISA results for rabbit experiments H1 to H4[a]

Expt/ Group	Rabbit #s	Prime	Prime		Protein Boost		Adjuvant	O.D. (2 weeks post-2nd boost: screen at 1:100)*						
		DNA	V1V2	Scaffold	V1V2	Scaffold		cV2 92TH023	V1V2/ A244-tags	V1V2/ 1086 -tags	V1V2/ 92TH023-gp70	V1V2/ YU2-1FD6	V1V2/ ZM109-1FD6	V1V2/ZM109 (N160K)-1FD6
Clade								E	E	C	E	B	C	C
H1/1	1-5	ZM109F-opt gp120	—	—	Case A2 (B)	gp70	IFA	1.3	1.5	1.9	2.9	0.4	1.6	1.6
H1/2	6-10	ZM109F-opt gp120	—	—	1086	Tags	IFA	0.6	1.9	2.4	1.9	0.2	1.0	1.1
H1/3	11-15	ZM109F-opt gp120	—	—	ZM109	1FD6	IFA	1.0	0.9	0.5	1.1	1.5	2.3	2.9
H1/4	16-20	ZM109F-opt gp120	—	—	CAP45	1FD6	IFA	0.9	1.0	1.0	1.5	1.2	2.0	2.5
H1/5	21-25	ZM109F-opt gp120	—	—	ZM109 gp120		IFA	1.9	1.6	1.8	2.4	0.2	2.4	3.1
H2/1	26-28	ZM109F-opt gp120	—	—	ZM109 gp120		IFA	2.5	1.4	1.3	1.7	0.1	1.9	2.3
H2/2	29-32	ZM109F-opt gp120	—	—	ZM53	2J9C	IFA	0.6	0.8	0.8	0.4	1.0	2.1	1.7
H2/3	33-36	ZM109F-opt gp120	—	—	1086	Fc	IFA	0.5	1.3	2.8	1.3	0.1	0.7	0.4
H2/4	37-41	ZM109F-opt gp120	—	—	ZM109	1FD6-Fc	IFA	1.0	1.2	1.2	0.6	1.9	2.8	2.8
H2/5	42-46	ZM109F-opt gp120	—	—	ZM109	TTB	IFA	0.4	0.4	0.4	0.2	1.0	1.9	1.8
H2/6	47-50	ZM109F-opt gp120	—	—	ZM109	1FD6 x CTB	IFA	1.1	0.8	0.7	0.6	1.0	1.8	1.8
H3/1	51-55	ZM109F-opt gp120	—	—	ZM53	2F5K	IFA	1.1	1.4	2.1	1.8	1.8	2.5	3.0
H3/2	56-60	ZM109F-opt gp120	—	—	ZM109	1FD6-Fc	IFA	0.7	0.8	0.7	1.0	1.7	1.8	2.7
H3/3	61-65	ZM109F-opt gp120	—	—	ZM109	Fc	IFA	1.3	1.1	1.6	1.6	1.8	2.8	3.2
H3/4	66-70	ZM109F-opt gp120	ZM53	2J9C	ZM53	2J9C	IFA	1.3	1.7	2.3	1.4	2	3.0	3.2
H3/5	71-75	ZM109F-opt gp120	ZM109	TTB	ZM109	TTB	IFA	0.8	0.7	1.2	0.8	1.7	3.0	3.2
H4/1	76-80	ZM109F-opt gp120	ZM53	2J9C	ZM53	2J9C	Alum	0.7	1.6	0.9	0.9	1.0	2.0	1.8
H4/2	81-85	ZM109F-opt gp120	ZM53	2J9C	ZM53	2J9C	IFA	1.3	2.2	1.2	1.9	1.7	3.3	2.8
H4/3	86-90	—	ZM53	2J9C	ZM53	2J9C	IFA	0.7	1.8	0.6	1.2	1.5	3.2	2.8
H4/4	91-95	ZM109F-opt gp120	ZM53	2J9C	A244	2J9C	IFA	0.5	1.1	0.3	0.5	1.0	2.5	2.3

≤0.12	0.13 - 0.99	1.0 -1.99	2.0- 2.99	≥ 3.0

[a] The reactivity of prebleed sera diluted 1:100 against each of the antigens gave an optical density reading of ≤0.12.

exception of group 3 from experiment H4 [H4/3]) (Table 3) were primed with codon-optimized DNA of gp120 from clade C strain ZM109F, as indicated in Table 3; the timing of each immunizing dose is shown in Table 2. Five groups of animals were coimmunized with DNA and protein, followed by protein-only boosting (Table 3).

Characterization of the Ab response. Rabbits were bled prior to and 2 weeks after each immunization. For all rabbits, the sera drawn 2 weeks after the last boost were used at 1:100 dilutions to screen by ELISA for reactivity against various V1V2 antigens. The results in Table 3 show the mean optical densities (ODs) from all animals in each group when sera were tested for Ab reactivity with a variety of antigens carrying the V1V2 region from various clades and in various configurations. Thus, $cV2_{92TH023}$ (clade E), $V1V2_{1086}$-tags (clade C), $V1V2_{A244}$-tags (clade E), and $V1V2_{92TH023}$-gp70 (clade E) were present in an unconstrained configuration since the mobility of the V1V2 region was not limited by the structure of, or the placement in, the scaffold. In contrast, the flexibility of the V1V2 regions present in $V1V2_{YU2}$-1FD6 (clade B), $V1V2_{ZM109}$-1FD6 (clade C), and the N160K mutant of $V1V2_{ZM109}$-1FD6 was constrained by its placement in the 1FD6 scaffold (49). The reagents used here have been described previously (16, 30, 49, 50). Reactivity of prebleed sera, diluted 1:100, against each of these antigens gave OD readings of $\leq$0.12. Sera drawn from animals after two boosts with all of the immunization regimens were reactive above the prebleed background levels with essentially all antigens, indicating that all of the various V1V2-scaffold proteins were immunogenic and induced Abs that were highly cross-clade reactive with epitopes displayed by diverse V1V2 regions. The pattern evident from the heat map of ELISA reactivities shown in Table 3 indicates that the strongest responses were in animals immunized with immunogens bearing constrained V1V2 epitopes (displayed on scaffolds 1FD6, 2J9C, 1FD6-Fc, TTB, and 2F5K) and tested with ELISA antigens displaying V1V2-constrained epitopes [$V1V2_{YU2}$-1FD6, $V1V2_{ZM109}$-1FD6, and $V1V2_{ZM109(N160K)}$-1FD6]. The *P* value using a one-tailed Student *t* test was 0.0035 when the OD values derived using the three V1V2-constrained ELISA antigens and sera of animals immunized with V1V2-constrained immunogens were compared to the OD values of ELISAs derived from sera of rabbits immunized with V1V2-unconstrained immunogens (displayed as part of gp70, tags, gp120, or Fc). The Ab response was also stronger when protein immunogen was included with the DNA priming immunization and not just in the boost (H2/2 versus groups H3/4 and H4/2, where the protein immunogen was $V1V2_{ZM53}$-2J9C [$P = 0.001$ by the one-tailed Mann-Whitney test]). This reproduces previously published data (51).

As shown previously, V1, V2, and V3 form the apex of the envelope trimer on the surface of virions and infected cells (52–54). Three categories of Abs have been described which are specific for different epitope regions in V1V2 (55, 56), as follows.

(i) V2p Abs target a linear epitope represented by a peptide from the C strand of V2, which assumes a helical structure when bound to V2p MAbs. Only a few such human V2p-specific MAbs have been described, and these appear to be quite limited in their neutralizing ability but are able to mediate ADCC (16, 57). V2p-specific Abs were generated in the studies described herein as rabbit sera bound to the cyclic peptide $cV2_{92TH023}$ (Table 3), a reagent which is recognized only by V2p-specific monoclonal Abs (MAbs) (Fig. 1).

(ii) V2i Abs target a highly conformational epitope that includes the integrin binding site at residues 179 to 181 (55, 56). Many V2i MAbs have been described, and serum V2i Abs exist in the majority of infected individuals (58–60); these polyclonal and monoclonal Abs do not react with V2-derived peptides but are highly cross-reactive with monomeric gp120 from diverse strains and clades (61) as well as with V1V2-scaffolded proteins such as $V1V2_{CaseA2}$-gp70 (Fig. 1) (58, 59). Binding of serum Abs to $V1V2_{CaseA2}$-gp70 was correlated with the reduced HIV infection rate in RV144 vaccinees, demonstrating that the RV144 vaccine regimen induced V2i-like Abs (13, 28, 30). Several of the reagents used to interrogate rabbit immune sera were able to bind to V2i MAbs, as shown in Fig. 1. $V1V2_{ZM109}$-1FD6, for example, binds to several V2i MAbs, but V2i MAbs do not bind to the $cV29_{2TH023}$ peptide. As shown in Table 3, immune sera from all rabbits bind strongly to $V1V2_{ZM109}$-1FD6.

(iii) V2q, also designated as V2 apex Abs, such as MAbs PG9 and CH01 (62, 63) preferentially target a V1V2 peptidoglycan which is part of the structure created by the quaternary interaction of the three V1V2 domains in the Env trimer (62, 64). These MAbs mediate broad and potent neutralization, and, for avid binding, these V2q MAbs require the presence of the glycan at position N160 (62, 64). To determine if V2q-specific Abs were induced in immunized rabbits, we tested their ability to bind to an N160K mutant of $V1V2_{ZM109}$-1FD6. As shown in Table 3, Abs in rabbit serum bound as well to $V1V2_{ZM109(N160K)}$-1FD6 as to $V1V2_{ZM109}$-1FD6. In addition, rabbit immune serum did not mediate neutralization (see below). These data indicate that the Abs induced in the rabbits immunized with V1V2 scaffolds did not have the immunochemical or functional characteristics of the potent and broadly neutralizing V2q Abs.

To further characterize the responses to the various immunogens used here, Ab reactivities were compared when the boosting regimen used gp120 protein instead of various V1V2-scaffold immunogens. The data are shown in Fig. 2, in which immune sera from all animals in experiments H2, drawn 2 weeks after the second boost, were tested for reactivity with $V1V2_{ZM109}$-1FD6. As expected, the prebleed sera showed no reactivity, while sera from all immunized rabbits reacted with $V1V2_{ZM109}$-1FD6. Of particular import is that the levels of Abs in the animals boosted with various V1V2-scaffold immunogens (with the exception of $V1V2_{1086}$-Fc) were comparable to the levels of Abs achieved in rabbits boosted with the $gp120_{ZM109}$ protein. Notably, the reciprocal of the geometric mean titer for 50% neutralization ($GMT_{50}{}^{-1}$) in $gp120_{ZM109}$-boosted animals (Fig. 2, group H2/1) was 280 compared to the $GMT_{50}{}^{-1}$ value of 838 for animals boosted in the same experiment with $V1V2_{ZM109}$-1FD6-Fc ($P = 0.03$, by a one-tailed Mann-Whitney analysis). There appeared to be little if any contribution of anti-scaffold Abs to the $GMT_{50}{}^{-1}$ values (data not shown), which is consistent with the relatively small size and expected poor immunogenicity of the scaffolds; e.g., the molecular mass of 1FD6 is 6.3 kDa, that of TTB is 12.5 kDa, that of 2F5K is 10.6 kDa, and that of 2J9C is 12.9 kDa.

Comparison of the efficacies of various immunization regimens. To compare a series of variables, radar plots were developed to indicate the mean levels in animal sera of Abs against six different antigens (Fig. 3). Each of the graphs (Fig. 3A to E) shows the mean OD values of all animals in each of two groups immunized to test a single variable. In all cases, the response of one group was

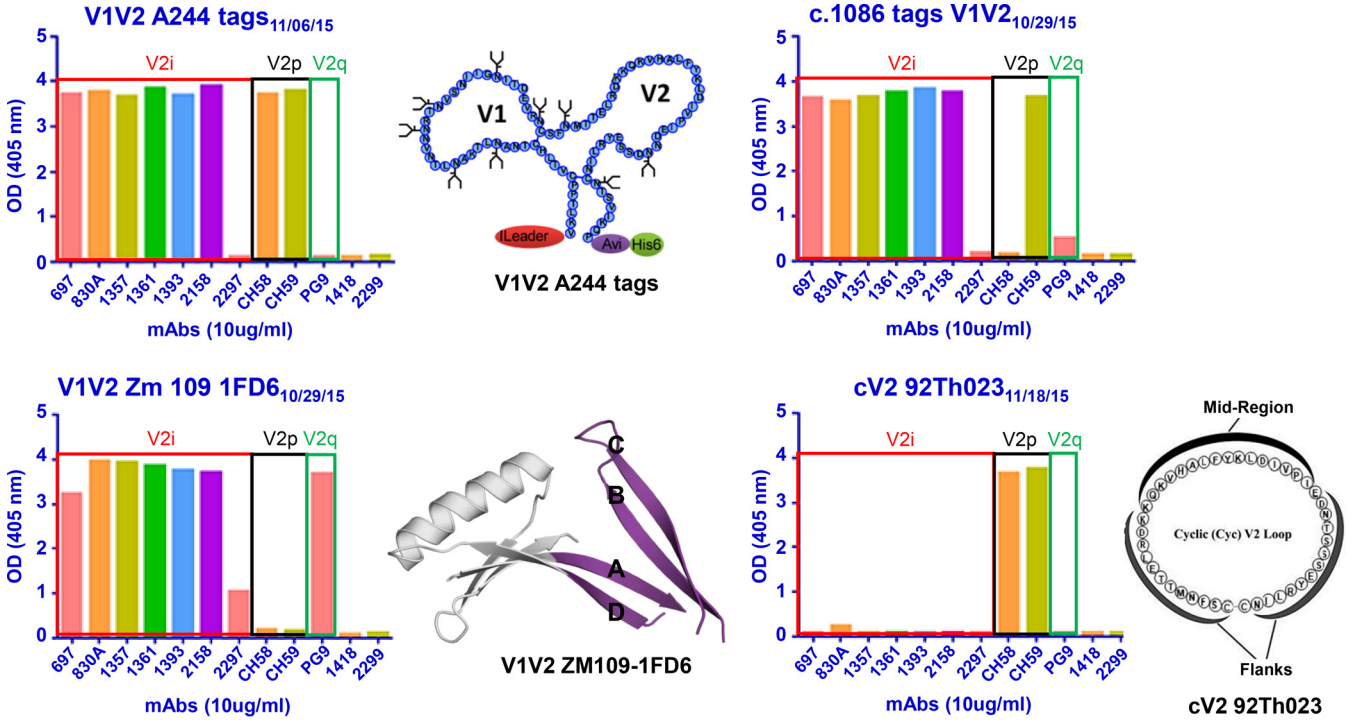


FIG 1 Monoclonal antibody (MAb) reactivity against various V1V2 scaffolds. The optical densities (ODs) of the ELISA reactions between several human MAbs (used at dilutions of 10 μg/ml) and various V1V2-scaffold antigens are shown. MAbs are designated specific for the V2i, V2p, or V2q epitope, as defined in the text. Diagrams of $V1V2_{A244}$-tags, of $V1V2_{ZM109}$-1FD6, and of $cV2_{92TH023}$, which were used as antigens in these ELISAs, are shown. For $V1V2_{ZM109}$-1FD6, V1V2 is shown in purple, and 1FD6 is in gray. The four strands, A, B, C, and D, of V1V2 are marked. Negative controls consisted of human MAb 1418, specific for parvovirus B19, and human MAb 2299, specific for gp70 of murine leukemia virus.

consistently stronger to all six antigens. For example, animals that were coprimed with gp120 DNA plus a V1V2-scaffold protein (either $V1V2_{ZM109}$-TTB or $V1V2_{ZM53}$-2J9C) and that received two boosts with the same V1V2-scaffold protein gave consistently stronger Ab responses against six V1V2 antigens than animals primed with gp120 DNA alone and boosted with a V1V2-scaffold protein (Fig. 3A and B).

The specifics of each panel in Fig. 3 are provided in Fig. 3F,

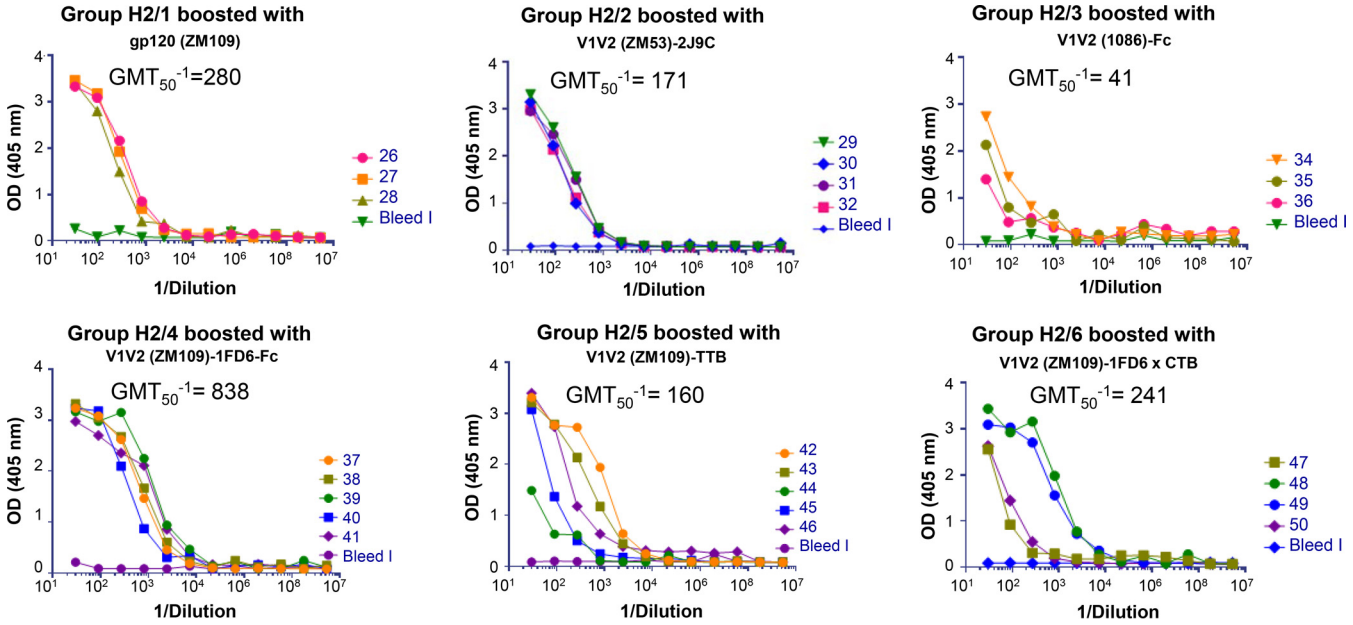
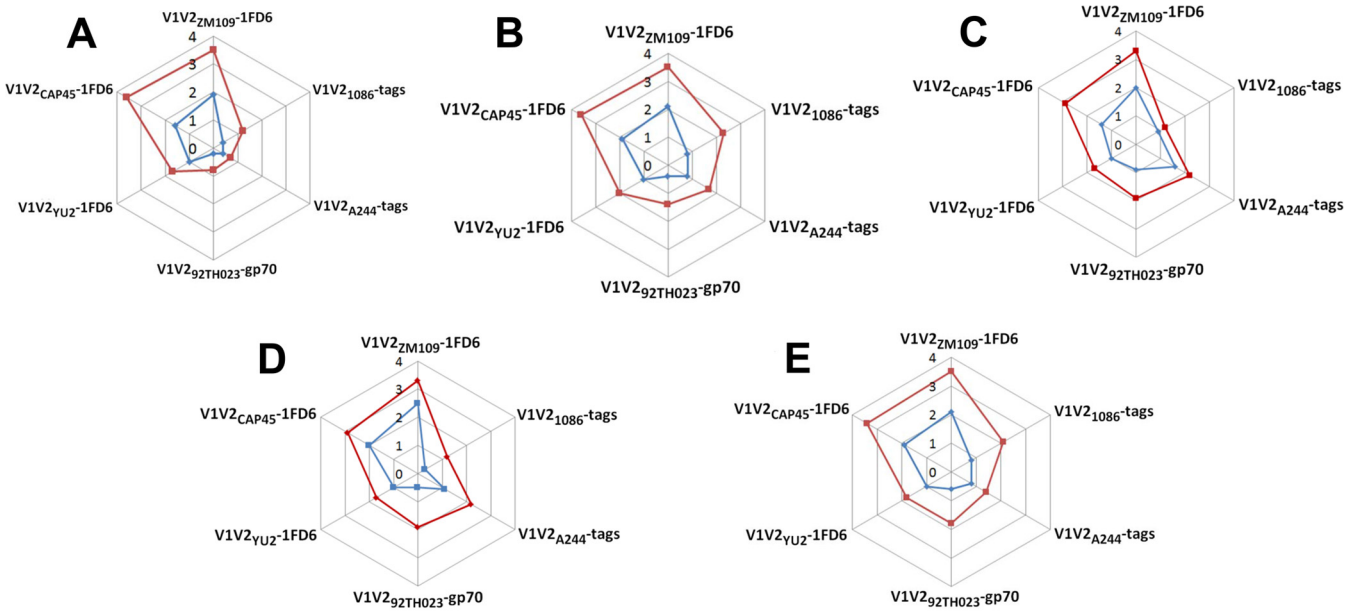


FIG 2 Titration of immune rabbit sera against $V1V2_{ZM109}$-1FD6. Pools of prebleed sera (bleed I) from each group or serum drawn 2 weeks after the last boost from individual animals (numbered 26 to 50) in each of the six groups of animals immunized as part of experiment H2 (Table 3) were titrated in ELISAs.



F

Figure	Group	Prime (x3)*	Boost (x2)*	Adjuvant*	GMT_{50}^{-1} vs. $V1V2_{ZM109}$-1FD6	p-value (one-tailed Mann Whitney test)
3A	H2/5; blue	DNA gp120/ZM109	V1V2/ZM109-TTB	IFA	160	0.041
3A	H3/5; red	DNA gp120/ZM109 + V1V2/ZM109-TTB	V1V2/ZM109-TTB	IFA	959	
3B	H2/2; blue	DNA gp120/ZM109	V1V2/ZM53-2J9C	IFA	171	0.030
3B	H3/4; red	DNA gp120/ZM109 + V1V2/ZM53-2J9C	V1V2/ZM53-2J9C	IFA	2246	
3C	H4/1; blue	DNA gp120/ZM109 + V1V2/ZM53-2J9C	V1V2/ZM53-2J9C	Alum	37	0.029
3C	H4/2; red	DNA gp120/ZM109 + V1V2/ZM53-2J9C	V1V2/ZM53-2J9C	IFA	149	
3D	H4/4; blue	DNA gp120/ZM109 + V1V2/ZM53-2J9C	V1V2/A244-2J9C	IFA	22	0.022
3D	H4/2; red	DNA gp120/ZM109 + V1V2/ZM53-2J9C	V1V2/ZM53-2J9C	IFA	149	
3E	H2/2; blue	DNA gp120/ZM109	V1V2/ZM53-2J9C	IFA	171	0.001
3E	H3/1; red	DNA gp120/ZM109	V1V2/ZM53-2F5K	IFA	1412	

FIG 3 Radar graphs showing antibody responses in sera obtained from immunized rabbits drawn 2 weeks after the second boost. (A to E) Mean OD values are shown for sera diluted 1:100. (F) Details of the immunization of each group of animals, along with the geometric mean reciprocal half-maximal titers of sera from individual rabbits in each group (GMT_{50}^{-1}), are shown for ELISAs in which $V1V2_{ZM109}$-1FD6 was used as the coating antigen. *, underlining denotes the difference in the immunization regimens between the two experiments in each pair compared.

which also shows the GMT_{50}^{-1} values against one of the antigens, $V1V2_{ZM109}$-1FD6, for each group. For example, for the data shown in Fig. 3A where $V1V2_{ZM109}$-TTB was used as the protein immunogen, the GMT_{50}^{-1} levels for the two different priming strategies (DNA plus protein versus DNA alone) were 959 and 160, respectively; similarly, for the data shown in Fig. 3B where $V1V2_{ZM53}$-2J9C was used as the protein immunogen, the GMT_{50}^{-1} levels for the two different priming strategies (DNA plus protein versus DNA alone) were 2,246 and 171, respectively (Fig. 3F). The *P* values generated with a one-tailed Mann-Whitney test are shown to be significant for each of the pairs compared (Fig. 3F).

Animals that were coprimed three times with DNA and the $V1V2_{ZM53}$-2J9C protein and then boosted with $V1V2_{ZM53}$-2J9C using incomplete Freund's adjuvant (IFA) gave stronger responses than animals receiving the same immunization regimen using alum as an adjuvant (Fig. 3C). The GMT_{50}^{-1} values of the sera from the animals in these groups, when titrated against

TABLE 4 Duration of the antibody response during and after priming with $gp120_{ZM109}$ DNA and boosting with $gp120_{ZM109}$ (experimental group H1/5)[a]

BLEED	Week		ZM233 gp120	V1V2/ YU2-1FD6	V1V2/ ZM109-TTB	V1V2/Case A2-gp70	V3 ConsC
I	0	Pre-bleed	0.17	0.09‡	0.12	0.09	0.08
II	2	2wp1p*	0.36	0.09	0.19	0.10	0.09
III	4	2wp2p	2.21	0.09	0.23	0.24	0.17
IV	6	2wp3p	2.87	0.09	0.36	0.64	0.24
V	10	6wp3p	2.02	0.10	0.26	0.57	0.11
VI	12	2wp1b	2.98	0.31	0.84	1.62	0.44
VII	16	2wp2b	2.97	0.34	1.06	2.07	0.52
VIII	18	4wp2b	3.03	0.34	1.01	2.31	0.72
IX	21	7wp2b	2.86	0.27	0.91	1.81	0.38

[a] Values are the mean ODs at 405 nm of five sera tested at 1:100. Data from 36 prebleed sera in various experiments were used to calculate a cutoff value (mean + 3 standard deviations) of ≥0.14. The results during priming are shown in bleeds I to V, and those during and after boosts are represented in bleeds VI to IX. Abbreviations for the prime-boost regimen use the following form: 2wp1p, 2 weeks post-1st prime; 2wp1b, 2 weeks post-1st boost. V3 ConsC, V3 consensus C peptide.

$V1V2_{ZM109}$-1FD6, were 149 and 37 for the IFA and alum groups, respectively (Fig. 3F).

Animals that were coprimed with DNA plus $V1V2_{ZM53}$-2J9C scaffold protein and boosted with the homologous $V1V2_{ZM53}$-2J9C scaffold protein gave a better response than animals primed with DNA plus the $V1V2_{ZM53}$-2J9C scaffold protein and boosted with the heterologous $V1V2_{A244}$-2J9C scaffold protein (Fig. 3D). The GMT_{50}^{-1} values for the homologous versus heterologous prime and boosting regimens were 149 and 22, respectively (Fig. 3F).

The $V1V2_{ZM53}$-2F5K protein immunogen gave a stronger response than the $V1V2_{ZM53}$-2J9C immunogen, as shown in Fig. 3E. The GMT_{50}^{-1} values for titration against $V1V2_{ZM109}$-1FD6 for these two groups were 1,412 and 171, respectively (Fig. 3F).

Durability of the Ab response. To determine the durability of the immune response elicited with various immunogens and vaccine regimens, selected groups of rabbits were housed for up to 76 weeks and bled at various times after each immunization. The kinetics and the duration of the Ab responses against various antigens are shown for three groups of animals in Tables 4, 5, and 6. When $gp120_{ZM109F}$ DNA was used alone for the priming immunization, Abs reacted poorly with all antigens except $gp120_{ZM233}$ (Table 4, experimental group H1/5). After a boost with $gp120_{ZM109F}$ protein, reactivity of $gp120_{ZM233}$-directed Abs increased and remained strong, and Abs to $V1V2_{CaseA2}$-gp70 were significantly elevated although reactivity to other antigens remained weak. Antibody levels began to wane at week 21, which was 11 weeks after the last boost. In contrast, when DNA plus V1V2-scaffold immunogens were used for the three priming doses, followed by boosting with V1V2-scaffold proteins carrying the homologous or heterologous V1V2 regions in the epitope scaffold boosting immunogen (Tables 5 and 6, respectively), robust responses were generated against both V1V2 antigens and gp120 after the second or third priming dose. Antibody levels to the V1V2-scaffold antigens increased after the protein boosts. Rabbits were maintained and bled periodically for the longest period of time in the experiment where rabbits received DNA plus $V1V2_{ZM53}$-2J9C as the prime immunization and $V1V2_{ZM53}$-2J9C for the boost (Table 5, experiment H3/4). The Ab response to V1V2 antigen constructs from clades B ($V1V2_{YU2}$-1FD6) and C ($V1V2_{ZM109}$-TTB) were clearly enhanced by the protein boosts and were maintained at peak activity through weeks 26 to 36, which corresponded to 16 to 26 weeks after the last protein boost; after that there was a very gradual decline. Ab responses were still detectable at week 76, which was 66 weeks after the last boost. As seen in Table 3, V1V2-specific Abs reacted most strongly when the V1V2 domain was held in a constrained position by the scaffold proteins (in this case $V1V2_{YU2}$-1FD6 and $V1V2_{ZM109}$-TTB) as opposed to antigens that harbored the V1V2 domain in an unconstrained configuration ($gp120_{ZM233}$ and $V1V2_{CaseA2}$-gp70).

The data in Tables 4, 5, and 6 also indicate that the Ab response is focused on V1V2. Antibodies reactive with a V3 consensus C peptide were induced, as expected, in the rabbits immunized with $gp120_{ZM109}$ DNA and $gp120_{ZM109}$ protein (Table 4), but in animals primed with DNA $gp120_{ZM109}$ plus V1V2 scaffolds and boosted with the V1V2 scaffolds, V3 Abs were detectable only at levels just above background and only during the immunization with the priming gp120 DNA (Tables 5 and 6). Further data are presented in Fig. 4 indicating that the Ab responses were focused on V1V2 to the exclusion of other gp120 epitopes. Here, the sera of animals primed with DNA and boosted with the V1V2-scaffold protein immunogen $V1V2_{ZM109}$-1FD6 (Fig. 4A) or $V1V2_{ZM53}$-2J9C (Fig. 4D) are shown to react after the boost with only $V1V2_{ZM109}$-1FD6 and not with the C5 and V3 peptides, which are two of the most immunogenic epitopes in gp120 (65, 66). In contrast, several of the animals that were primed with DNA and boosted with the $gp120_{ZM109}$ protein reacted with either the C5 and/or the V3 peptide (Fig. 4B and C). Additionally, the data

TABLE 5 Duration of antibody response during and after priming with DNA plus $V1V2_{ZM53}$-2J9C and boosting with $V1V2_{ZM53}$-2J9C (experimental group H3/4)[a]

BLEED	Weeks		ZM233 gp120	V1V2/ YU2-1FD6	V1V2/ ZM109-TTB	V1V2/CaseA2-gp70	V3 ConsC
I	0	Pre-bleed	0.15	0.09	0.10	0.09	0.08
II	2	2wp1p	0.62	0.13	0.14	0.09	0.13
III	4	2wp2p	2.38	1.02	1.00	0.24	0.28
IV	6	2wp3p	2.66	1.99	1.78	0.45	0.26
V	12	2wp1b	1.67	2.47	2.29	0.38	0.14
VI	16	2wp2b	1.27	2.61	2.32	0.58	0.15
VII	20	6wp2b	1.05	2.55	2.37	0.45	0.14
VIII	26	12wp2b	1.51	2.48	2.42	0.38	0.14
IX	36	22wp2b	1.39	2.14	1.87	0.27	0.12
X	57	43wp2b	1.28	1.67	1.70	0.23	0.12
XI	61	47wp2b	1.15	1.54	1.43	0.26	0.10
XII	69	55wp2b	1.94	2.15	1.81	0.19	0.14
XIII	76	62wp2b	1.63	1.93	1.79	0.19	0.14

[a] Values are the mean ODs at 405 nm of five sera tested at 1:100. Data from 36 prebleed sera in various experiments were used to calculate a cutoff value (mean + 3 standard deviations) of ≥0.14. The results during priming are shown in bleeds I to IV, and those during and after boosts are represented in bleeds V to XIII (indicated by the black line). Abbreviations for the prime-boost regimen use the following form: 2wp1p, 2 weeks post-1st prime; 2wp1b, 2 weeks post-1st boost. V3 ConsC, V3 consensus C peptide.

shown in Fig. 4 indicate that the gp120 DNA priming induced only weak Ab responses, and these responses were against C5, V1V2, and/or V3. The subsequent protein boost was responsible for the induction of the strong Ab responses, and in the case of the animals receiving the V1V2-scaffold protein immunogens, the prime-induced weak responses to C5 and V3 had waned completely (Fig. 4A and D). Thus, Ab responses induced by the rationally designed V1V2-scaffold protein immunogens focused the

TABLE 6 Duration of the antibody response during and after priming with DNA + $V1V2_{ZM53}$-2J9C and boosting with $V1V2_{A244}$-2J9C (experimental group H4/4)[a]

BLEED	Weeks		ZM233 gp120	V1V2/ YU2-1FD6	V1V2/ ZM109-TTB	V1V2/Case A2-gp70	V3 ConsC
I	0	Pre-bleed	0.13	0.09	0.09	0.09	0.09
II	10	2wp3p	2.00	2.51	2.4	0.56	0.15
III	16	2wp1b	1.38	2.15	2.29	0.39	0.11
IV	20	2wp2b	1.19	1.66	2.49	0.40	0.11
V	22	4wp2b	1.20	1.72	2.48	0.42	0.10
VI	24	6wp2b	1.08	1.38	2.63	0.39	0.11
VII	35	17wp2b	1.07	0.73	2.16	0.24	0.10
VIII	42	24wp2b	1.33	0.81	2.48	0.34	0.12
IX	50	32wp2b	1.45	0.55	2.26	0.23	0.10

[a] Values are the mean ODs at 405 nm of five sera tested at 1:100. Data from 36 prebleed sera in various experiments were used to calculate a cutoff value (mean + 3 standard deviations) of ≥0.14. The results during priming are shown in bleeds I and II, and those during and after boosts are represented in bleeds III to IX (indicated by the black line). Abbreviations for the prime-boost regimen use the following form: 2wp1p, 2 weeks post-1st prime; 2wp1b, 2 weeks post-1st boost. V3 ConsC, V3 consensus C peptide.

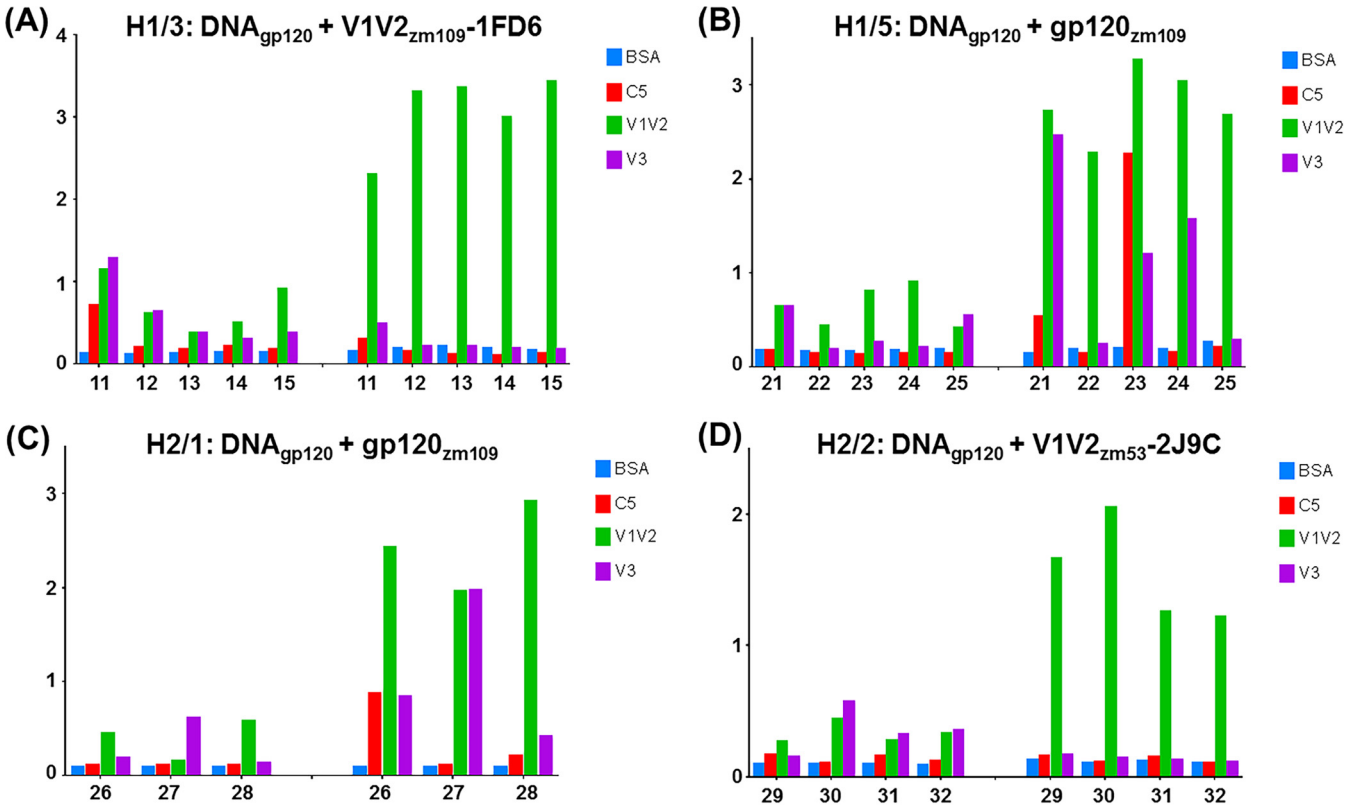
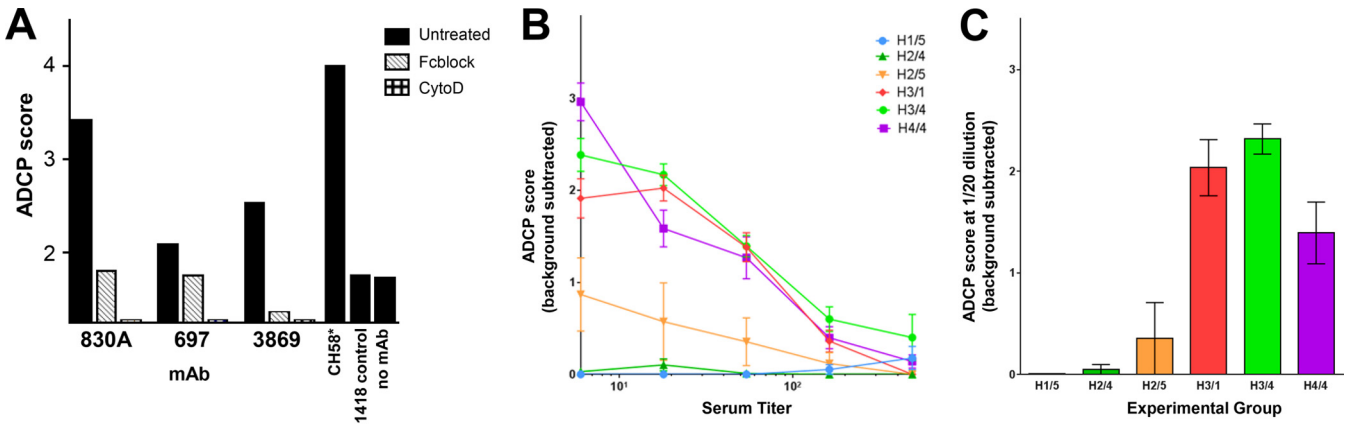


FIG 4 Specificity of the Ab response. The average OD values of replicates from ELISAs are shown on the *y* axis for sera from individual animals tested for reactivity against BSA, C5 peptide, V1V2$_{ZM109}$-1FD6, and a consensus C V3 peptide. The responses are shown for sera diluted 1:100 from each animal (numbered on the *x* axis). The left half of each graph shows the results with sera collected 2 weeks after the third priming immunization. The right half of each graph shows the responses obtained with sera collected 2 weeks after the second boost.

Ab response on the V1V2 region to the exclusion of other Env antigens.

Biologic function of Abs induced by V2-targeting vaccines. V2i-specific MAbs have little to no neutralizing activity (59), and while there was a correlation between reduced risk of infection in RV144 vaccinees and the level of V1V2 binding Abs, there was no correlation with serum neutralizing activity (15). Thus, we did not expect the immune sera from the rabbits immunized in these studies to display neutralizing activity. To test for neutralizing activity of immune rabbit sera, a modified, more sensitive version of the TZM.bl assay was used (67) in which virus and sera were incubated together for 24 h prior to application to cells. Indeed, there was no neutralizing activity when sera from the five animals in one of the best groups (H3/4; primed with gp120$_{ZM109F}$ DNA plus V1V2$_{ZM53}$-2J9C and boosted with V1V2$_{ZM53}$-2J9C) were tested against BaL.26, YU2, and SF162 (data not shown).

Since increasing attention has been focused on Fc-dependent Ab functions, we chose to test the ability of the serum Abs from immunized rabbits to mediate phagocytosis (12, 18, 24, 38). We used the assay that was first described by Ackerman et al. (48) to assess the phagocytic activity of THP-1 cells incubated with various HIV Env-specific human MAbs bound to beads coated with biotinylated gp120$_{ZM53}$. Figure 5A shows the Ab-dependent cellular phagocytosis (ADCP) scores generated using the optimized assay described in the Materials and Methods section. Robust phagocytosis was achieved with beads coated with gp120$_{ZM53}$ and then bound to V2i MAb 830A or 697 or to the V3-specific MAb 3869. This activity is shown to be Fc dependent, as illustrated by the ability of cytochalasin D and human Fc receptor blocking reagent (Fig. 5, Fcblock) to inhibit phagocytosis. The V2p-specific MAb CH58 was also shown to be capable of mediating ADCP of beads coated with the cV2 peptide. This optimized assay was next used to test the phagocytic activity of serum from each rabbit in several groups of immunized animals. Immune sera were each titrated from a dilution of 1:6 to 1:486 for phagocytic activity against beads coated with V1V2$_{ZM109}$-1FD6, and the mean ADCP score for each group of rabbits at each of five serum dilutions is plotted in Fig. 5B. Similar levels of phagocytic activity were detected in sera from rabbits in three of the six groups tested: H3/1 (primed with gp120 DNA and boosted with V1V2$_{ZM53}$-2F5K), H3/4 (primed with gp120 DNA plus V1V2$_{ZM53}$-2J9C protein and boosted with V1V2$_{ZM53}$-2J9C), and H4/4 (primed with gp120 DNA plus V1V2$_{ZM53}$-2J9C protein and boosted with V1V2$_{A244}$-2J9C). In contrast, little or no activity was detected in sera from animals in H1/5 (primed with gp120 DNA and boosted with gp120 protein), H2/4 (primed with DNA and boosted with V1V2$_{ZM109}$-1FD6-Fc), and H2/5 (primed with DNA and boosted with V1V2$_{ZM109}$-TTB). A similar pattern was found for sera tested at a dilution of 1:20 derived from the same six groups of animals and tested using beads coated with V1V2$_{YU2}$-1FD6 (Fig. 5C). It is particularly striking that boosting with gp120 protein does not induce Abs with phagocytic activity (group H1/5) while three groups boosted with V1V2$_{ZM53}$-2F5K, V1V2$_{ZM53}$-2J9C, or V1V2$_{A244}$-2J9C displayed strong activity in this assay; this denotes



D

Expt/ Group	Prime			Boost		OD vs. $V1V2_{ZM109}$-1FD6
	DNA	V1V2	Scaffold	V1V2	Scaffold	
H1/5	ZM109-opt gp120	-	-	ZM109 gp120		2.4
H2/4	ZM109-opt gp120	-	-	ZM109	1FD6-Fc	2.8
H2/5	ZM109-opt gp120	-	-	ZM109	TTB	1.8
H3/1	ZM109-opt gp120	-	-	ZM53	2F5K	3.4
H3/4	ZM109-opt gp120	ZM53	2J9C	ZM53	2J9C	3.5
H4/4	ZM109-opt gp120	ZM53	2J9C	A244	2J9C	2.5

FIG 5 Phagocytosis assay with monoclonal antibodies (MAbs) and rabbit immune sera. The ADCP assay was performed and reported as ADCP scores as described in Materials and Methods. (A) Beads were coated with either $gp120_{ZM53}$ and then incubated with the designated MAb (V2i-specific MAb 830A or 697 or V3-specific MAb 3869) or with cV2 peptide and incubated with V2p-specific MAb CH58. Cells that had been preincubated for 30 min at 37°C with either 30 μl of FcR blocking agent (Fcblock; Miltenyi Biotec) or 10 μl of 10 μg/ml cytochalasin D (CytoD; Sigma) or were untreated and then added to the bead-MAb complexes. Controls consisted of $gp120_{ZM53}$-coated beads without bound MAb (no MAb) or incubated with MAb 1418, specific for B19 parvovirus (1418 control). (B) Titration of phagocytic activity in rabbit immune sera using beads coated with $V1V2_{ZM109}$-1FD6. Data shown are the means of values from individual rabbits in each group ± standard errors of the means. (C) ADCP scores for rabbit immune sera diluted 1:20 when beads coated with $V1V2_{YU2}$-1FD6 were used. Data shown are the means from individual rabbits in each group ± standard errors of the means. (D) Immunization regimen used for each of the six rabbit groups studied and the mean OD determined from ELISAs with a 1:100 dilution of serum from each individual rabbit in each group tested for reactivity with $V1V2_{ZM109}$-1FD6.

a significant qualitative difference in the Abs induced by trimeric V1V2-scaffold immunogens as opposed to gp120 protein, dimeric immunogens, or pentameric immunogens. It is noteworthy that there is no correlation between phagocytic activity with $V1V2_{ZM109}$-1FD6-coated beads and Ab reactivity of the same serum in ELISAs against $V1V2_{ZM109}$-1FD6 (Fig. 5B and D). These data are not contradictory in that phagocytosis is a function of an Fc-mediated activity (as shown in Fig. 5A) while ELISA reactivity reflects binding of Abs to the antigen via the Fab fragment.

DISCUSSION

The experiments described here were designed to focus a vaccine-induced Ab response on the V1V2 region of the HIV-1 gp120 envelope glycoprotein since Abs to this region have been associated with reduced rates of HIV and SIV infection (12, 13, 18, 28, 30, 31, 32, 33, 34, 68). The results show that when different immunization regimens employing gp120 DNA and various V1V2 protein scaffold immunogens were used, the V1V2 region was targeted by the Ab response, with little or no induction of Abs to other Env epitopes. The response was durable, with Abs detectable for ≥1 year after the last immunization. The vaccine-induced Abs were (i) highly cross-reactive with the V1V2 regions and gp120 molecules derived from diverse strains and clades, (ii) directed at linear and conformational epitopes in V2, and (iii) able to mediate phagocytosis, a function that has been correlated with protection from HIV, SIV, and SHIV as well as other viral diseases (12, 18, 24, 38). The specificities of the vaccine-induced rabbit Abs were similar to those of human-derived V2i-specific MAbs on the basis of their ELISA reactivities with various V1V2-scaffold immunogens (Table 3 and Fig. 1), their inability to neutralize viruses, and their ability to mediate Fc-dependent phagocytosis (Fig. 5).

The rationale for using an epitope-specific approach to vaccine design was based on studies in the literature indicating that targeting specific epitopes of infectious organisms could protect against infection by various pathogens, an approach termed reverse vaccinology (69). For example, targeting the factor H-binding protein of *Neisseria meningitides* with an experimental vaccine prevented meningococcal meningitis and sepsis in mice (70), and targeting the site Ø of the fusion glycoprotein of respiratory syncytial virus (RSV) elicited high levels of RSV-specific Abs with neutralizing activity (71). Moreover, focusing the immune response on the V3 region of HIV gp120 with V3-scaffold immunogens induced cross-clade neutralizing Abs and recapitulated the binding and biologic activity of V3-specific human MAbs (35, 36).

Here, we describe a successful reverse vaccinology approach using immunogens that target the V1V2 portion of gp120. This study and the successful reverse vaccinology studies cited above stand in marked contrast to several unsuccessful attempts to use epitope scaffold immunogens to elicit polyclonal responses designed to recapitulate the specificity and function of broadly neutralizing MAbs that target HIV sites of vulnerability (72–76).

It has been suggested that nonneutralizing mechanisms, such as phagocytosis, neutrophil activation, and ADCC, are likely to play a role in both HIV prevention and reservoir-eliminating therapeutic approaches (77, 78). This is supported by the RV144 clinical trial where a correlate of reduced infection was found with various Fc-mediated activities but not with neutralization (9–18). In addition, a myriad of studies have shown an association between Fcγ-dependent Ab functions and reduced infection with SIV and SHIV (22, 24, 32, 33, 38).

The mechanism by which Abs mediate various Fc-dependent antiviral functions is the subject of ongoing studies. The protective role of HIV Abs in binding to Fc receptors was established in passive immunization experiments (79). In addition, the nature of Fc-glycan structures was shown to selectively promote Fc-effector functions independent of Ab specificity for HIV epitopes, and particular Ab glycan structures were associated with enhanced ADCC as well as ADCP activity (80). Further studies have indicated that Fcγ receptor-mediated activity is associated with preferential engagement of activating, but not inhibitory, Fcγ receptors (81). Thus, biologic functions of nonneutralizing Abs are affected by many factors, including isotype, subtype, affinity for Fc receptors, and ability to activate complement—all of which are functions of the Fc rather than the Fab fragment of immunoglobulins (82). It is notable, therefore, that in targeting the immune response to V1V2, we have induced Abs that, as expected on the basis of previous data (59), have no neutralizing activity but mediate ADCP. Notably, there was no phagocytic activity in the sera of rabbits immunized with gp120 DNA and gp120 protein (experimental group H1/5) whereas the immune sera we tested from animals immunized with selected gp120 DNA and V1V2-scaffold proteins (experimental groups H3/1, H3/4, and H4/4) displayed this activity (Fig. 5), indicating that there was a qualitative difference between the immune responses induced by gp120 and those induced by V1V2-scaffold immunogens.

The induction of ADCP by immunogens V1V2-2F5K and V1V2-2J9C, in contrast to the failure to induce ADCP with gp120 protein, may bear on the differing conformations of these antigens. V1V2 is presented by the 2F5K and 2J9C scaffolds as an apical trimeric structure whose conformation is constrained by their insertion in these scaffolds (47). In contrast, the V1V2 region in gp120 is presented as a monomeric, unconstrained domain. The V1V2-2J9C design was based on the V1V2 structure as observed in complex with V2q MAb PG9 (49), while the V1V2-2F5K design was based on the V1V2 structure in complex with V2i MAb 830A (37). The geometry of the trimeric configuration (position and orientation with respect to the trimer axis) of the V1V2 domains in both designs was guided by the low-resolution cryo-electron microscopy (EM) structure of the SOSIP trimeric spike in complex with MAb PG9 (53) before the higher-resolution structure became available (54).

In summary, we have used the structure of V1V2 epitopes recognized by various human MAbs that target the V1V2 region of gp120 to design V1V2-scaffold immunogens which induced Abs in rabbits that recapitulated the specificity and functional activity of human V2i MAbs. The vaccine-induced rabbit serum Abs were focused on the V1V2 region, were cross-clade reactive, mediated Ab-dependent phagocytosis, and were detectable ≥1 year after the last immunizing dose. The data demonstrate the success of a reverse vaccinology approach to HIV vaccine design and the potential for specifically targeting sites of vulnerability with rationally designed immunogens.

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